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## Extinguished Cocaine Cues Increase Drug Seeking When Presented Simultaneously With a Non-Extinguished Cocaine Cue

David N. Kearns<sup>1</sup> and Stanley J. Weiss

American University

### Abstract

**Background**—Previous research with non-drug reinforcers has shown that simultaneously presenting (compounding) an extinguished cue with another cue formerly associated with the same reinforcer can increase rates of cue-controlled behavior. The present study investigated whether an extinguished cocaine cue would energize cocaine seeking when presented simultaneously with another cocaine cue. This study also investigated whether extinction could be enhanced by subjecting an extinguished cocaine cue to further extinction after administration of reinstating injections of cocaine.

**Methods**—Rats were first trained to self-administer cocaine in the presence of three different cues. Then, one of the cues was subjected to the standard extinction treatment. Another cue was subjected to a modified extinction treatment where additional extinction sessions were preceded by non-contingent cocaine injections. The third cue was not extinguished.

**Results**—The cue subjected to standard extinction ceased to control cocaine seeking when presented alone, but significantly increased cocaine seeking when compounded with the non-extinguished cocaine cue. The cocaine cue subjected to the modified extinction treatment also significantly increased cocaine seeking occasioned by the non-extinguished cocaine cue.

**Conclusions**—Extending results of previous studies involving non-drug stimuli, the present study showed that extinguished cocaine cues can enhance cocaine seeking when compounded with other cocaine cues. These results illustrate the persistence of drug cues in controlling behavior despite extinction and highlight the need for developing treatments that eliminate this residual energizing capacity that survives extinction.

### Keywords

extinction; drug cues; cocaine; self-administration; cue exposure; stimulus compounding; rats

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<sup>1</sup>Corresponding author: David N. Kearns, Psychology Department, American University, 4400 Massachusetts Ave NW, Washington, DC 20016, Phone: 202-885-1725, FAX: 202-885-1023, dk0085a@american.edu.

#### Contributors

Both authors designed the experiment. David Kearns performed the experiment, analyzed the data, and wrote the first draft of the manuscript. Both authors contributed to and have approved the final manuscript.

#### Conflict of Interest

Both authors declare that they have no conflicts of interest.

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## 1. Introduction

Drug cues, such as people, places, or things (e.g., paraphernalia) associated with drug use, play an important role in driving drug abuse and addiction. For example, drug cues elicit craving for the drug (Childress et al., 1999; Volkow et al., 2006), activate the same brain reward circuitry that is activated by the drug itself (Volkow et al., 2006, 2008), and contribute to relapse after abstinence (Grusser et al., 2004; Kosten et al., 2006; Sinha and Li, 2007). An intervention that reduces the power of drug cues could help to improve treatment outcomes.

Extinction has been used in such an effort. Extinction is the presentation of a conditioned cue without the reinforcer (or unconditioned stimulus; US) with which the cue was previously paired. For example, cue-exposure therapy is an extinction-based treatment where drug users are repeatedly exposed to drug cues without the drug (Drummond et al., 1995). Experiencing the cues in the absence of the drug should theoretically break (or inhibit) the cue-drug association and thereby reduce the ability of the drug cues to drive drug-related behavioral or neurological responses. While there have been a handful of cue-exposure studies reporting promising results (Loeber et al., 2006; Rohsenow et al., 2001), the outcomes of most cue-exposure studies have been less encouraging than expected (e.g., Marissen et al., 2007; for review, see Conklin and Tiffany, 2002).

Basic learning research on extinction may suggest potential reasons for the disappointing results of cue-exposure therapy (Conklin and Tiffany, 2002). For example, experiments with rats have found that a non-drug cue (i.e., a stimulus associated with food or shock) that appeared to be behaviorally silent after extensive extinction still energized responding when presented simultaneously with another cue associated with the same reinforcer or US (Kearns and Weiss, 2005; Hendry, 1983; Reberg, 1972; Rescorla, 2006). Reberg (1972) performed the original experiment demonstrating this effect. In that study, rats were trained on a procedure where two conditioned stimuli (CSs) were each separately paired with electric shock. The CSs came to elicit a fear response that suppressed rats' lever pressing for food. Then, one of the CSs was subjected to extensive extinction (i.e., the CS was repeatedly presented without shock). The extinguished CS no longer had any suppressive effect on lever pressing by the end of the extinction phase. But when this extinguished CS was presented simultaneously with a still-active CS (i.e., a CS not subjected to extensive extinction), there was greater suppression of lever pressing than when the still-active CS was presented alone. This suggests that extinguished cues retain residual excitatory associative properties even after extinction appears to have eliminated their control over behavior. This latent excitation is revealed when the extinguished cue is compounded with another cue that has an excitatory history. Kearns and Weiss (2005) replicated and extended Reberg's (1972) finding by showing that compounding (i.e., simultaneously presenting) extinguished food-associated discriminative stimuli ( $S^D$ s) also reactivated these cues' control over behavior.

It is possible that drug cues extinguished as part of cue-exposure therapy are similarly reactivated when they are presented simultaneously with non-extinguished, still-active drug cues encountered outside of the treatment setting. If so, this might help explain why cue-exposure therapy has not been more effective. The primary goal of the present study was to model this situation in rats by compounding an extinguished  $S^D$  that previously occasioned cocaine self-administration with another cocaine  $S^D$  that was not extinguished. A second goal was to attempt to develop a treatment that would more effectively extinguish cocaine cues and eliminate potential residual excitatory properties that might survive standard extinction. This treatment was based on recent basic learning research showing that extinction can be enhanced by increasing the excitation present during additional non-reinforced exposure to previously extinguished cues (Janak and Corbit, 2011; Leung and

Westbrook, 2008; Rescorla, 2006). These previous studies used either stimulus compounding (Janak and Corbit, 2011; Rescorla, 2006) or spontaneous recovery (Lueng and Westbrook, 2008) to reactivate the previously extinguished cues prior to subjecting them to additional extinction. Drug self-administration research has shown that non-contingent presentation of the drug is one of the most effective ways of reinstating extinguished cocaine seeking (de Wit and Stewart, 1981; Shaham et al., 2003; Shalev et al., 2002). Therefore, the present study used non-contingent injections of cocaine to reinstate cocaine seeking during additional non-reinforced exposures to the previously extinguished cocaine cues.

The effectiveness of this modified extinction treatment was evaluated on a stimulus compounding test, where the treated cue was presented simultaneously with a non-extinguished cocaine cue. Previous research has shown that stimulus compounding tests are very sensitive measures of the associative properties conditioned to discriminative cues (Weiss, 1978; Weiss and Schindler, 1985). If the modified treatment is effective in enhancing extinction, it would be expected that a cue subjected to it would be less likely (than a cue subjected to standard extinction) to energize responding when compounded with a non-extinguished cocaine cue. Such an outcome could suggest a way to improve extinction-based drug abuse treatments. For example, after standard extinction of cocaine cues, individuals might be given (under medical supervision) small amounts of cocaine or a similar drug with lower abuse potential (e.g., methylphenidate) prior to additional non-reinforced exposures to the cues.

## 2. Materials and methods

### 2.1. Subjects

Eight adult male Long-Evans rats served as subjects. Rats were individually housed in plastic cages with wood chip bedding and metal wire tops. Subjects were maintained at 85% of their free-feeding weights (approximately 350–450 g) throughout the experiment by feeding them approximately 15 g of rat chow following training sessions. Rats had unlimited access to water in their home cages. The colony room where the rats were housed had a 12-h light:12-h dark cycle with lights on at 08:00 h. Training sessions were conducted 5–7 days per week during the light phase of the light:dark cycle. Throughout the experiment, rats were treated in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 1996) and all procedures were approved by American University's Institutional Animal Care and Use Committee (IACUC).

### 2.2. Apparatus

Training took place in six operant chambers. Each chamber was 20 cm high, 23 cm long, and 18 cm wide and had aluminum front and rear walls, white translucent plastic side walls, a clear plexiglass ceiling, and a grid floor. A response lever and food trough were located on the front wall of the chamber. A tone stimulus (4000 Hz and 80 dB) was delivered through a speaker mounted 21.5 cm above the chamber and inside the sound-attenuation chest. A light stimulus was provided by two 15-cm, 25-W, 120-VAC tubular light bulbs located 10 cm outside of the chamber's side walls. The light bulbs were operated at approximately 110 VAC. A fan (Sunon, model # KDE2412PMB3-6A) mounted on top of the chamber was used to provide a "fan" stimulus that this lab has used in previous studies (Lombas et al., 2008a, 2008b). When the fan was activated, air blew through ventilation holes in the ceiling of the chamber. Operation the fan also produced some vibration and associated running hum. The square frame that housed this fan measured approximately 12 cm × 12 cm and was positioned over the response lever. The fan was operated at 15 VDC. Each chamber was housed inside a sound attenuation chest (Weiss, 1970) that also had a continuously operating ventilation fan (separate from the stimulus fan). A shielded houselight mounted on the

ceiling (and near the rear wall) of the sound attenuation chest provided a low level of continuous illumination during all sessions. The illumination produced by this houselight was just enough to make the rat barely discernible when in the chamber. Experimental procedures were controlled by Med-Associates software (Med-PC, St. Albans, VT) running on a PC located in an adjacent room.

Cocaine (National Institute on Drug Abuse, Bethesda, MD) in saline solution at a concentration of 2.56 mg/ml was infused at a rate of 3.19 ml/min by 10-ml syringes driven by Harvard Apparatus (South Natick, MA) or MED Associates (St. Albans, VT) syringe pumps located outside of the sound-attenuation chests. Tygon tubing extended from the 10-ml syringes to a 22-gauge rodent single-channel fluid swivel and tether apparatus (Alice King Chatham Medical Arts, Hawthorne, CA) that descended through the ceiling of the chamber. Cocaine was delivered to the subject through Tygon tubing that passed through the metal spring of the tether apparatus. This metal spring was attached to a plastic screw cemented to the rat's head to reduce tension on the catheter.

### 2.3. Procedure

**2.3.1. Lever-press acquisition**—To facilitate later lever pressing for cocaine, rats were first trained for 2 sessions to lever press for food pellets. Food pellets were available for lever pressing on a fixed-ratio 1 (FR-1) schedule and were also presented non-contingently every 120 s on average (range: 90–150 s) if a lever press was not made. These two sessions lasted approximately 60 min.

**2.3.2. Surgery**—Rats were then surgically prepared with chronic indwelling jugular vein catheters, using a modification of the procedure originally developed by Weeks (1962). In brief, under ketamine (60 mg/kg) and xylazine (10 mg/kg) anesthesia, approximately 3 cm of Silastic tubing (0.044mm i.d., 0.814mm o.d.) was inserted into the right jugular vein. This Silastic tubing was connected to 8 cm of vinyl tubing (Dural Plastics; 0.5mm i.d., 1.0mm o.d.) that was passed under the skin around the shoulder and exited the back at the level of the shoulder blades. The vinyl tubing was threaded through a section of Tygon tubing (10 mm long, 4 mm diameter) that served as a subcutaneous anchor. Six stainless steel jeweler's screws were implanted in the skull, to which a 20-mm plastic screw was cemented with dental acrylic. Catheters were flushed daily with 0.1 ml of a saline solution containing 1.25 U/ml heparin and 0.08 mg/ml gentamycin.

**2.3.3. Cocaine self-administration**—After 1 week of recovery in the homecage, rats were trained to self-administer cocaine on a 4-component multiple schedule. During light, tone, or fan  $S^D$  components, 1.0 mg/kg cocaine infusions were available for lever pressing according to an FR-1 schedule. These  $S^D$  components alternated with  $S^A$  components where all stimuli were absent and lever pressing did not result in cocaine. Each of the three types of  $S^D$  component (light, tone, or fan) were equally likely to follow an  $S^A$  component, with the restriction that there were no more than two consecutive  $S^D$  components of the same type. Initially,  $S^D$  components lasted 900 s and  $S^A$  components lasted 300 s. Over sessions, the FR value operative in  $S^D$  components was gradually increased from 1 to 10. Once a rat displayed regular responding on the FR-10 schedule, a variable-ratio (VR) 10 schedule was introduced in place of the FR-10 schedule during light, tone, and fan components. Now, an average of 10 (range: 1–15) lever presses were required for each cocaine infusion.  $S^D$  and  $S^A$  components lasted 300 s on average (range: 240–360 s). After 2 sessions on this VR-10 procedure, the dose of self-administered cocaine was lowered to 0.5 mg/kg/infusion where it remained for the rest of the experiment.

After 2 sessions of training with the 0.5 mg/kg/infusion dose, rats were put on the terminal baseline schedule. As previously, cocaine was available on a VR-10 schedule during light, tone, and fan  $S^D$  components, but was not available during  $S^A$  components when these stimuli were absent. Now, the lengths of  $S^D$  and  $S^A$  components were reduced to 180 s on average (range: 120–240 s). In addition, a 60-s response correction contingency operated during the final 60 s of each  $S^A$  component. According to this contingency, a 60-sec  $S^A$ -termination clock was reset to zero by a response. Thus, at least 60 s had to pass from the time of the last lever press in an  $S^A$  component to the onset of the next  $S^D$  component. This contingency was designed to reduce response rates during  $S^A$  components. The length of the response-correction contingency was increased as necessary for individual rats up to as long as 300 s if responding persisted during  $S^A$  components. Rats were trained on this terminal baseline schedule for a minimum of 8 sessions and until (1) response rates in all  $S^D$  components averaged over two sessions were at least 3 times faster than rates in  $S^A$ , and (2) tone and light response rates were within 20% of each other. Catheter patency was confirmed at the end of self-administration training by aspirating blood through the catheter or by observing rapid ataxia (loss of motor control within 10 s) after infusion of 0.1 ml of a saline solution containing 0.3 mg ketamine and 0.4 mg xylazine.

**2.3.4. Extinction Phase 1**—All rats were then given 3 sessions of extinction of the light and the tone. The light and the tone were subsequently counterbalanced in their roles as the stimuli subjected to the modified extinction treatment or the standard extinction treatment. The light and the tone were selected for these roles because our laboratory has much experience with these stimuli and our goal was to equate the treated stimuli as much as possible in terms of salience. The fan was not subjected to extinction, because this stimulus would serve as the non-extinguished excitator on subsequent stimulus compounding tests. During the extinction Phase 1 sessions, the light and the tone were each presented 6 times. Each tone or light component lasted 180 s and was followed by a 180-s period where these stimuli were absent. Tone and light were equally likely to follow each absence component, with the restriction that there were no more than 2 consecutive  $S^D$  components of the same type (light or tone). Lever pressing did not produce cocaine infusions at any time and the response correction contingency in  $S^A$  was discontinued.

**2.3.5. Extinction Phase 2**—The light and the tone were then each subjected to 3 additional extinction sessions. For one of the  $S^D$ s, designated Stimulus X, these extinction sessions were preceded by an i.p. injection of 15 mg/kg cocaine. This dose was chosen because previous research has shown it to be an effective reinstater of extinguished cocaine seeking (Keiflin et al., 2008a). For the other  $S^D$ , designated Stimulus Y, these additional extinction sessions were preceded by an i.p. injection of saline (equivolume to 15 mg/kg cocaine). The light and the tone were counterbalanced in their roles as Stimulus X and Stimulus Y. Only one stimulus type was presented within a session. Stimulus X and Stimulus Y extinction sessions alternated. Half of the subjects received these sessions in the order X,Y,X,Y,X,Y and the other half in the order Y,X,Y,X,Y, X. I.p. injections were administered 5–10 minutes prior to placing the rat in the chamber. During an extinction session, the to-be-extinguished stimulus was presented 8 times for periods that lasted 180 s and were separated by 120-s periods where all stimuli were absent. Lever pressing was not reinforced at any time. To minimize the possibility that the i.p. cocaine injection administered prior to Stimulus X extinction sessions would also reinstate responding during Stimulus Y extinction sessions, a session of context exposure alone was administered on the day after each Stimulus X or Stimulus Y extinction session. During these context exposure sessions, the animal was placed in the chamber for 40 min without any stimulus presentations. This was designed to reduce any potential excitation conditioned to the context by the i.p. injection of cocaine, since previous research has shown that reinstatement

depends on context excitation levels (Bouton and Bolles, 1979a). Context exposure sessions were not preceded by injections of cocaine or saline. Thus, during Phase 2, there were a total of 12 sessions: 3 Stimulus X extinction sessions with each followed by a session of exposure to the chamber alone and 3 Stimulus Y extinction sessions with each followed by a session of exposure to the chamber alone.

**2.3.6. Stimulus Compounding Test 1**—After the final extinction Phase 2 session, the first of two stimulus compounding tests was administered. This test consisted of 6 presentations each of the fan alone (F), the fan-plus-light compound (FL), and the fan-plus-tone compound (FT). These test stimuli were presented in the order F, FL, FT, FL, FT, F, FT, F, FL, F, FT, FL, FL, F, FT, FT, FL, F. Thus, each block of 3 test stimuli contained 1 presentation of each stimulus and the ordinal position of each stimulus within a block was balanced over blocks. Each test condition lasted 75 s and was followed by 75-s periods where all stimuli were absent. Lever presses were not reinforced at any time during the test.

**2.3.7. Stimulus Compounding Test 2**—When the first compounding test ended, rats remained in the chamber and were given a fan alone component during which they could self-administer 0.5 mg/kg cocaine infusions on an FR-1 schedule. The onset of this fan component coincided with the non-contingent administration of a single 0.5 mg/kg cocaine infusion. Once rats self-administered 3 infusions, this fan component ended. After a 75-s period where all stimuli were absent, the second compounding test began. The procedural details of this test were the same as those described above for the first stimulus compounding test. As previously, lever presses were not reinforced at any time during the test. Catheter patency was determined following this test using the methods described previously.

## 2.4. Data Analysis

For all statistical tests,  $\alpha = 0.05$ . Separate repeated measures ANOVAs were performed on response rates during the final two self-administration sessions, all extinction Phase 1 sessions, all extinction Phase 2 sessions, the last extinction Phase 1 and first extinction Phase 2 sessions, and the two stimulus compounding tests. Paired-sample t-tests were performed where appropriate following significant ANOVAs. Paired-sample t-tests were also used to compare the fan-plus-X and fan-plus-Y compounds in terms of the percentage of test blocks where responding was greater in the compound as compared to the fan alone.

## 3. Results

### 3.1. Self-administration

Rats had a mean of 28.5 ( $\pm 3.0$  SEM) total cocaine self-administration sessions, 19.8 ( $\pm 3.2$  SEM) of which were terminal baseline sessions. Response rates averaged over the final two sessions during fan, Stimulus X, and Stimulus Y were 8.5 ( $\pm 2.6$  SEM), 8.2 ( $\pm 3.0$  SEM), and 8.0 ( $\pm 2.8$  SEM) responses per min, respectively (see Figure 1). A repeated measures ANOVA indicated that there was no significant difference in response rates controlled by these  $S^D$ s ( $F[2,14] = 0.4, p > 0.6$ ). The mean response rate during the absence of all stimuli ( $S^\Delta$ ) was 2.0 ( $\pm 0.9$  SEM). A paired-samples t-test confirmed that the mean  $S^D$  response rate was significantly greater ( $t[7] = 3.5, p < 0.05$ ) than the  $S^\Delta$  response rate, which was to be expected since rats were trained to a criterion that required them to respond at least 3 times faster in the  $S^D$ s as compared to  $S^\Delta$ .

### 3.2. Extinction Phase 1

The left portion of Figure 2 presents mean ( $\pm$  SEM) responses per minute during Stimuli X and Y (tone or light, counterbalanced) during each component of the 3 sessions of the first

extinction phase. Mean response rates in each stimulus declined over sessions. A 2 X 3 (stimulus by session) repeated measures ANOVA performed on the Stimuli X and Y response rates indicated that there was a significant effect of session ( $F[2,14] = 12.0, p < 0.005$ ), but no significant effect of stimulus ( $F[2,7] = 0.0, p > 0.8$ ) and no significant interaction ( $F[2,14] = 0.6, p > 0.5$ ). The mean response rate during all-stimuli-off periods (not shown in figure) during extinction Phase 1 was  $0.3 (\pm 0.2 \text{ SEM})$  responses per min.

### 3.3. Extinction Phase 2

The right portion of Figure 2 presents mean ( $\pm \text{SEM}$ ) response rates during each component of each session during extinction Phase 2. The non-contingent cocaine injections administered prior to the start of Stimulus X sessions increased responding to Stimulus X. In contrast, responding to Stimulus Y remained low throughout this phase. As Figure 2 illustrates, mean response rates were higher during the Stimulus X component than during the corresponding Stimulus Y component in 24 out of 24 components during extinction Phase 2. On the final Stimulus Y extinction session, the mean response rate in Stimulus Y was  $0.1 (\pm 0.1 \text{ SEM})$  responses per min and half of the subjects did not make a single response in Stimulus Y during the entire session. The mean response rate in all-stimuli-off periods during Phase 2 extinction sessions was  $0.8 (\pm 0.2 \text{ SEM})$  responses per min.

To further test for reinstatement, an additional  $3 \times 2$  (stimulus by session) repeated measures ANOVA was performed on Stimulus X, Stimulus Y, and  $S^\Delta$  response rates from the final extinction Phase 1 session and the first extinction Phase 2 session. (The  $S^\Delta$  rate used for the Phase 2 session was the mean of the  $S^\Delta$  rates from the first Stimulus X and first Stimulus Y sessions in Phase 2). There were significant main effects of stimulus ( $F[2,14] = 11.9, p < 0.01$ ) and session ( $F[1,7] = 6.8, p < 0.05$ ) as well as a significant stimulus by session interaction ( $F[2,14] = 4.2, p < 0.05$ ). Subsequent paired-sample t-tests indicated that responding significantly increased over sessions for Stimulus X ( $t[7] = 2.5, p < 0.05$ ), but not for Stimulus Y ( $t[7] = .8, p > 0.45$ ) or for  $S^\Delta$  ( $t[7] = 1.3, p > 0.23$ ).

### 3.4. Stimulus Compounding Test 1

Figure 3a presents mean ( $\pm \text{SEM}$ ) response rates over the whole test during each test stimulus condition from the first stimulus compounding test. Figure 3b presents mean ( $\pm \text{SEM}$ ) cumulative responses over the 6 blocks of the test. One rat (j9) did not make any responses during this test and therefore was excluded from analysis. Stimulus Y, which controlled essentially no responding on its final extinction session, enhanced rates of cocaine-seeking behavior when compounded with the fan. Compounding Stimulus X with the fan similarly enhanced responding. A repeated measures ANOVA confirmed that response rates significantly differed over test conditions ( $F[2,12] = 5.9, p < 0.05$ ). Subsequent paired sample t-tests indicated that response rates during fan-plus-X and fan-plus-Y compounds were both significantly higher than in fan alone ( $t[6] \text{'s} > 2.6, p \text{'s} < 0.05$ ), but there was no difference between the two compound conditions ( $t[6] = 0.9, p > 0.3$ ). The mean response rate during all-stimuli-off periods during the first compounding test was  $0.3 (\pm 0.1 \text{ SEM})$  responses/min.

Table 1 presents individual subject data concerning block-by-block comparisons of each compound with the fan alone. For each subject and for each compound, the table presents the number of blocks where there was at least 1 response in the compound or the fan alone and the percentage of those blocks where responding in the compound was higher than in the fan alone. As the table shows, for both compounds there was greater responding to the compound than to the fan alone on approximately 70–75% of the test blocks (excluding those blocks where no responses were made). A paired samples t-test indicated that this percentage did not differ over compounds ( $t[6] = 0.6, p > 0.55$ ).

### 3.5. Stimulus Compounding Test 2

Figure 4a presents mean ( $\pm$  SEM) response rates over the whole test during each stimulus condition from the second compounding test, which followed a single fan component where rats self-administered 3 cocaine infusions. Figure 4b presents cumulative responses over the 6 blocks of the test. One rat (j11) was found to have a nonfunctional catheter after this test and therefore was excluded from analysis. As Figure 4 illustrates, presentation of Stimulus Y simultaneously with the fan increased rats' rate of cocaine-seeking by 100%, while Stimulus X only enhanced fan responding by approximately 67%. A repeated measures ANOVA indicated that there was a significant effect of test condition ( $F[2,12] = 6.4, p < 0.05$ ). Subsequent paired-sample t-tests revealed that responding in the fan-plus-Y compound was significantly higher than responding in fan alone ( $t[6] = 5.2, p < 0.005$ ), but the difference between responding in fan-plus-X and fan alone did not quite achieve significance ( $t[6] = 1.9, p = 0.11$ ). There was no significant difference between the two compound conditions ( $F[6] = 1.3, p > 0.2$ ). The mean response rate during all-stimuli-off periods during the second compounding test was  $0.1 (\pm 0.04)$  responses/min.

Table 2 presents the results of block-by-block comparisons of each compound with the fan alone for individual subjects. Similar to the results from Test 1, for each compound rats responded more to the compound than to the fan alone on approximately 70–75% of the test blocks. A paired-samples t-test indicated that this percentage did not significantly differ for the two compounds ( $t[6] = 0.4, p > 0.65$ ).

## 4. Discussion

The present experiment demonstrated that an extinguished cocaine cue, which did not control cocaine seeking when presented alone, was able to enhance cocaine seeking when presented simultaneously with another (non-extinguished) cocaine cue. This result replicates and extends those of previous studies involving non-drug reinforcers/USs showing that compounding extinguished cues leads to a reappearance of the behavior previously controlled by those cues (Hendry, 1982; Kearns and Weiss, 2005; Reberg, 1972; Rescorla, 2006). These studies and the present one show that a cue retains residual excitation even after extinction appears to have eliminated the power of the cue to control behavior on its own. This phenomenon, along with phenomena such as spontaneous recovery (e.g., Brooks and Bouton, 1994), reinstatement (Rescorla and Heth, 1975), context renewal (Bouton and Bolles, 1979b), and rapid reacquisition (Napier et al., 1992), shows that extinction does not erase the associations originally conditioned to a cue and that given the proper circumstances a behaviorally silent cue can exhibit a reappearance of its ability to influence behavior. Such dynamics may at least partially contribute to the discouraging results of extinction-based treatments such as cue-exposure therapy. For example, a drug cue subjected to extinction may show no control over behavior after cue exposure in the clinic, but is reactivated when compounded with a non-extinguished cue encountered in the user's normal environment. Consideration of this possibility, plus all of the other situations where extinguished drug cues might regain their control over behavior (e.g., spontaneous recovery, reinstatement, renewal), suggest that more effective extinction strategies must be developed if cue-exposure treatments are to be successful.

It is possible that the reactivation of the extinguished cues when compounded with the fan was due in part to context renewal processes. Research with non-drug reinforcers has shown that presenting an extinguished cue in a novel context different from the extinction context can lead to a reappearance of responding (AAB renewal; Bouton and Ricker, 1994). The fan-plus-X and fan-plus-Y compounds may have functioned as novel contexts different from the context in which X and Y were extinguished. It should be noted, though, that the fan in the present experiment was a discrete cue presented for relatively brief periods during the



test while in context renewal studies, contextual cues are typically present for the duration of the test session. Similarly, while fan-plus-X and fan-plus-Y would have been novel compounds, the rats had much exposure to the fan alone during initial self-administration training. Finally, in a similar stimulus compounding experiment using shock-paired cues rather than cocaine-paired cues, Hendry (1982) found that compounding an extinguished shock cue with a novel cue did not result in a reactivation of the shock cue, but compounding the shock cue with another extinguished shock cue did.

The present experiment investigated a treatment designed to enhance the extinction of a cue (Stimulus X) by administering reinstating injections of cocaine prior to further non-reinforced exposure to the cue. Though rats made over 10 times as many unreinforced responses during Stimulus X as they did during Stimulus Y in extinction Phase 2, Stimulus X still significantly enhanced cocaine seeking when compounded with the fan (that had not been subjected to extinction) during the first stimulus compounding test. Results from the second compounding test were largely similar. Though the difference between mean response rates in fan-plus-X vs. fan alone only approached significance ( $p = 0.11$ ), 6 out of 7 rats responded more during fan-plus-X components than during fan alone components on a majority of test blocks (see Table 2).

The Stimulus X extinction treatment employed here was based on previous non-drug experiments indicating that increasing the amount of excitation present during non-reinforced exposure to a cue facilitates extinction learning (Rescorla, 2000, 2006). The present experiment employed pre-session non-contingent cocaine injections in an effort to heighten excitation during the extinction of Stimulus X in the second extinction phase. These non-contingent cocaine injections were effective in reinstating cocaine seeking controlled by Stimulus X. But Rescorla (2006) has shown that the critical factor determining how much extinction a stimulus undergoes is not the number of responses controlled by that stimulus during extinction, but rather the amount of excitation present during non-reinforced exposure to the cue. This suggests that the non-contingent cocaine injections in the present experiment increased responding without increasing the amount of excitation experienced during subsequent non-reinforced exposure to the cue.

State-dependent extinction learning is another possible reason for the lack of effectiveness of the modified extinction treatment tested here. Delamater (2004) noted that this is a general problem for pharmacological treatments designed to enhance extinction. If drugs that are administered during extinction produce a discriminable internal cue, then any extinction learning that takes place may be specific to the drug state. Previous research has shown that a 15-mg/kg i.p. cocaine injection, like those administered during extinction Phase 2, can certainly produce a discriminable internal cue (Keiflan et al., 2008b). Thus, state-dependent extinction learning may partly explain why no enhancement of extinction was observed for Stimulus X on the first stimulus compounding test, which took place in the absence of cocaine.

On the transition from self-administration to extinction, rats' response rates dropped from approximately 8 responses per min (see Figure 1) to approximately 3 responses per min during the first component of the first extinction session (see Figure 2). It may have been expected that a rate closer to 8 responses per min would be observed early on in extinction. However, that rate reflects responding over the course of a 3-hr session where rats self-administered many cocaine infusions. On the first extinction session, rats lever pressed for the first time in a cocaine-free state. Previous studies have shown that that cocaine, whether self-administered or non-contingently administered, can have direct operant-response-rate-increasing effects in rats (Cantin et al., 2010; Jasyňa et al., 1998). For example, in a relevant recent study by Cantin et al. (2010), rats were trained on progressive ratio schedules to

respond for either cocaine or saccharin reinforcers. Rats responded at a much greater (~6 times faster) rate when cocaine was the reinforcer as compared to when saccharin was the reinforcer. However, when a 10-min post-reinforcer delay period was introduced to allow time for the direct effects of cocaine to dissipate somewhat, the rate of responding for cocaine dropped so much that there was no longer any difference between rates of responding for cocaine vs. saccharin. Further, when tested under extinction conditions, where the direct effects of cocaine were completely absent, the rate of response for cocaine was significantly lower than that for saccharin. Cantin et al. (2010) concluded that the direct effects of cocaine inflated self-administration response rates in their study. Similarly, the rate-increasing effects of self-administered cocaine likely also contributed to the relatively large difference observed in the present experiment between response rates during cocaine self-administration sessions and the initial extinction session. It is worth noting that comparably large drops in response rate have been observed in previous studies upon the transition to extinction from self-administration of cocaine on FR-10 (Valles et al., 2006) or VR-10 schedules (Kearns and Weiss, 2007).

The results of the present study show that extinguished cocaine cues can still energize cocaine-seeking behavior when they are presented simultaneously with non-extinguished cocaine cues. Improved extinction treatments that deal with this phenomenon are clearly necessary, given the importance of drug cues in driving drug craving and relapse (Childress et al., 1999; Kosten et al., 2006; Volkow et al., 2006). Basic research on extinction learning has shown that increasing excitation present during non-reinforcement of cues can enhance cue extinction (Rescorla, 2000, 2006). Future research that attempts to increase this excitation through means other than non-contingent administration of cocaine could prove to be informative. By drawing on these and other findings from basic research on extinction, an effective means of neutralizing the power of drug cues might be developed (Conklin and Tiffany, 2002).

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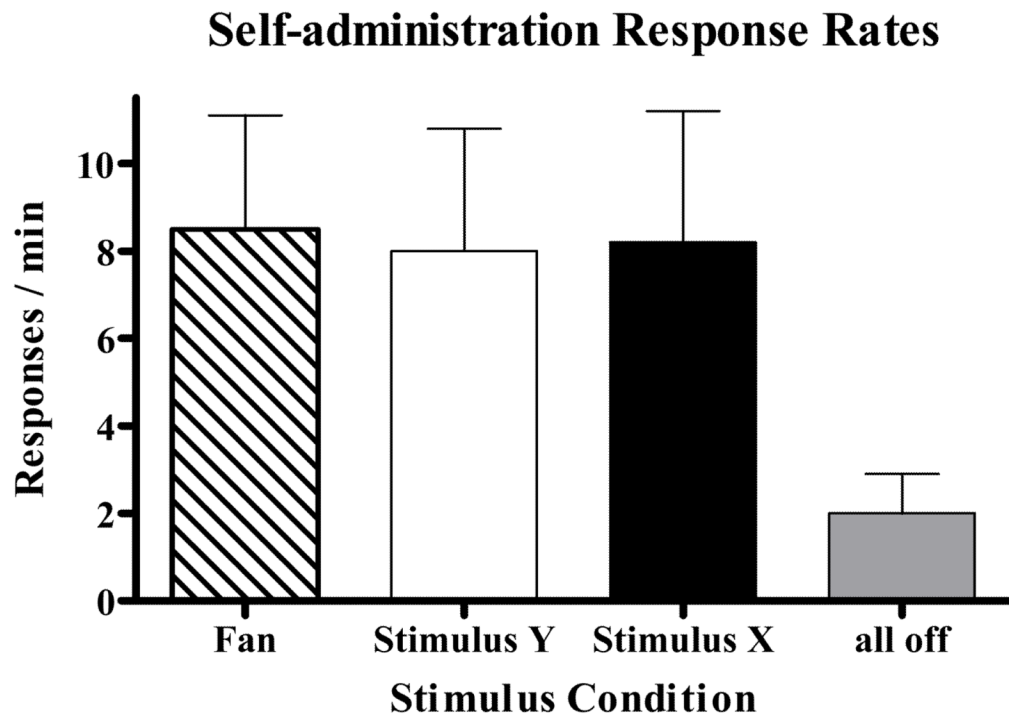
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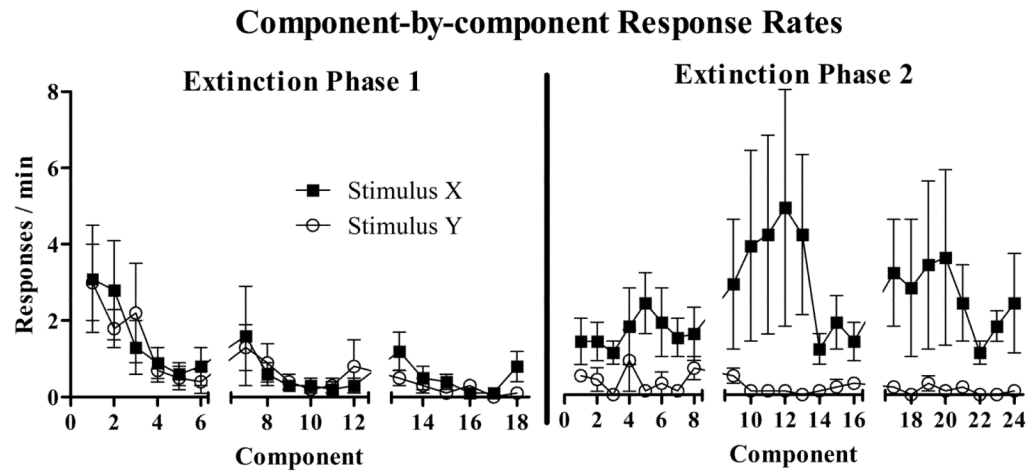
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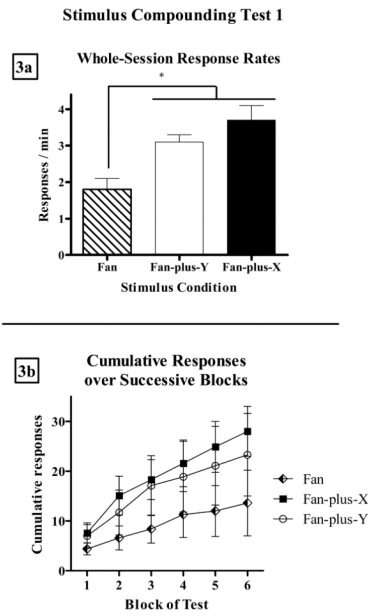
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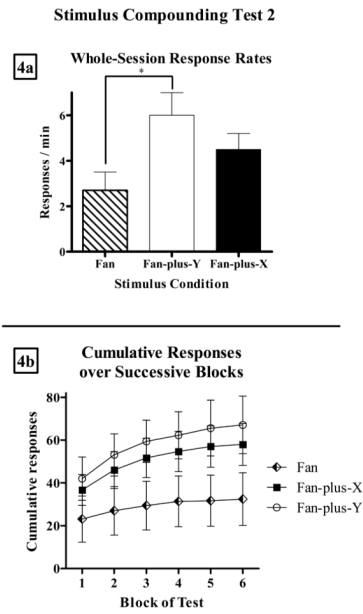
**Figure 1.** Mean ( $\pm$ SEM) response rates in the fan, Stimulus X, Stimulus Y, and all-stimuli-off components averaged over the last two self-administration sessions.



**Figure 2.** Mean ( $\pm$ SEM) response rates in Stimuli X (squares) and Y (circles) during each component of extinction Phase 1 (left of vertical line) and extinction Phase 2 (right of vertical line). There were 6 components per session during extinction Phase 1 and 8 components per session during extinction Phase 2.



**Figure 3.** (a) Mean ( $\pm$ SEM) whole-session response rates during fan alone (striped bar), fan-plus-Y (white bar), and fan-plus-X (black bar) on Stimulus Compounding Test 1. Asterisk indicates  $p < 0.05$ . (b) Mean ( $\pm$ SEM) cumulative responses in fan (diamonds), fan-plus-Y (circles), and fan-plus-X (squares) over the 6 blocks of the test.



**Figure 4.** (a) Mean ( $\pm$ SEM) whole-session response rates during fan alone (striped bar), fan-plus-Y (white bar), and fan-plus-X (black bar) on Stimulus Compounding Test 2. Asterisk indicates  $p < 0.05$ . (b) Mean ( $\pm$ SEM) cumulative responses in fan (diamonds), fan-plus-Y (circles), and fan-plus-X (squares) over the 6 blocks of the test.



**Table 1**

## Stimulus Compounding Test 1.

Subject	Fan-plus-X vs. Fan alone		Fan-plus-Y vs. Fan alone	
	# of blocks with at least one response in compound or fan alone	% of blocks where compound responding > fan alone responding	# of blocks with at least one response in compound or fan alone	% of blocks where compound responding > fan alone responding
j2	6	67	6	33
j7	4	100	3	100
j10	4	75	3	67
j11	3	67	3	33
j12	6	50	6	67
q16	4	100	3	100
q22	5	60	6	83
<b>Mean</b>	<b>4.6</b>	<b>74.0</b>	<b>4.3</b>	<b>69.0</b>
<b>SEM</b>	<b>0.4</b>	<b>7.3</b>	<b>0.6</b>	<b>10.6</b>

There were 6 total test blocks per test, with each block consisting of one presentation each of Fan alone, Fan-plus-X, and Fan-plus-Y components. Subject j9 did not make any responses on this test and therefore was excluded from data analysis.

**Table 2**

Stimulus Compounding Test 2.

Subject	Fan-plus-X vs. Fan alone		Fan-plus-Y vs. Fan alone	
	# of blocks with at least one response in compound or fan alone	% of blocks where compound responding > fan alone responding	# of blocks with at least one response in compound or fan alone	% of blocks where compound responding > fan alone responding
j2	6	67	6	67
j7	5	60	6	83
j9	3	67	4	100
j10	6	67	5	60
j12	6	33	6	67
q16	3	100	3	67
q22	6	100	5	80
<b>Mean</b>	<b>5.0</b>	<b>70.5</b>	<b>5.0</b>	<b>74.8</b>
<b>SEM</b>	<b>0.5</b>	<b>8.8</b>	<b>0.4</b>	<b>5.2</b>

There were 6 total test blocks per test, with each block consisting of one presentation each of Fan alone, Fan-plus-X, and Fan-plus-Y components. Subject j11 was found to have a nonfunctional catheter following this test and was therefore excluded from data analysis.